# CLAIMS

1. A separation matrix comprised of ligands coupled to the surfaces of a porous support, wherein the ligands provide at least one chemical gradient in the support.

2. A separation matrix according to claim 1, wherein the support comprises porous particles and the ligand gradient(s) extend between the centre and the exterior surface of each porous particle.

3. A matrix according to claim 1 or 2, wherein at least one gradient is a ligand density gradient formed by a changing density of ligands on the support.

4. A matrix according to claim 3, wherein two or more chemical gradients are present in the support and at least gradient is a ligand density gradient.

5. A matrix according to claim 3 or 4, wherein in the ligand density gradient(s), the ligand concentration increases towards the centre of the support.

6. A matrix according to claim 3 or 4, wherein in the ligand density gradient(s), the ligand concentration decreases towards the centre of the support.

7. A matrix according to any one of the preceding claims, which matrix is a chromatography matrix comprised of a plurality of essentially spherical particles, wherein each particle presents one or more gradient(s) perpendicular to the direction of the liquid flow applied in chromatography.

8. A matrix according to any one of the preceding claims, wherein at least one gradient is the result of varying pKa values of functional groups of the ligands present on the support.

9. A matrix according to any one of the preceding claims, wherein at least one gradient is the result of a varying net charge of the ligands present on the support.

10. A matrix according to any one of the preceding claims, wherein at least one gradient is a continuous and smooth gradient.

11. A matrix according to any one of the preceding claims, wherein the ligands present on the support provide at least two different functionalities.

12. A matrix according to claim 11, wherein said functionalities are selected from the group that consists of cation exchange ligands, anion exchange ligands, hydrophobic interaction chromatography (HIC) ligands, reversed phase chromatography (RPC) ligands, immobilised metal chelating ligands (IMAC), thiophilic ligands, and affinity ligands.

13. A matrix according to claim 11 or 12, wherein said at least two different functionalities are present on the same ligand.

14. A matrix according to claim 11, wherein the ligands present zwitterionic functionalities.

15. A matrix according to claim 11 or 12, wherein said at least two different functionalities are present on different ligand kinds, and each such ligand kind provides a separate chemical gradient within the support.

16. A chromatography column packed with a separation matrix comprised of ligands coupled to the surfaces of a porous support, wherein the ligands provide at least one chemical gradient within the support.

17. A chromatography column according to claim 16, wherein the support comprises essentially spherical porous particles and the ligands provide at least one chemical gradient between the centre and the exterior surface of each porous particle.

18. A chromatography column according to claim 16 or 17, wherein at least one gradient is a ligand density gradient formed by a changing density of ligands on the support.

19. A chromatography column according to any one of claims 16-18, which has been packed with a separation matrix according to any one of claims 1-15.

20. A method of providing a separation matrix comprising ligands coupled to the surfaces of a porous support, in which method at least one ligand density gradient is provided by solvent-controlled diffusion of at least one reagent into the porous support.

21. A method according to claim 20, wherein the solvent-controlled diffusion is obtained by contacting a first solvent comprising said reagent(s) with the support, in the pores of which a second solvent is present, said first and second solvents presenting different solubilities.

22. A method according to claim 21, wherein the first solvent is aqueous and the second solvent is organic.

23. A method according to claim 21, wherein the first solvent is organic and the second solvent is aqueous.

24. A method according to any one of claims 20-23, wherein the diffusion rate is controlled by adjusting one or more conditions selected from the group of temperature; air flow; solvent properties; and concentration and/or nature of functionalities.

25. A method according to claim 24, wherein the diffusion of reagent(s) is assisted by providing an essentially continuous air flow through the reaction mixture during the reaction.

26. A method according to any one of claims 20-25, wherein a ligand density gradient that decreases towards the centre of the support is provided by diffusion-controlled addition to the porous support of a reagent that comprises at least one functionality.

27. A method according to claim 26, wherein either the reagent or groups present on the surface of the porous support have been activated before the reaction.

28. A method according to any one of claims 20-25, wherein the support presents activated groups.

29. A method according to claim 28, wherein a ligand density gradient that increases towards the centre of the support is provided by diffusion-controlled addition to the porous support of a first reagent, which comprises deactivating groups, to deactivate in a controlled fashion some of the surface groups of the support, and subsequent addition of another reagent, which comprises at least one functionality, to couple said at least one functionality to the surface groups that have not been deactivated.

30. A method according to any one of claims 20-29, wherein the reagent comprises two different functionalities in a predetermined ratio to provide to different ligands in the separation matrix.

31. A method according to any one of claims 20-30, wherein the porous support comprises essentially spherical particles.

32. A method of preparing a separation matrix that comprises ligands coupled to the surfaces of a porous support, which method comprises the step of

(a) providing activatable groups on the surface of a porous support;

(b) activating said groups with an activation agent;

(c) reacting groups activated according to step b) with a compound which comprises at least one functionality;

wherein control of the reactivity in step (c) results in at least one chemical gradient within the support.

33. A method according to claim 32, wherein the reactivity is controlled by the concentration of the compound that comprises the functionalities in step (c).

34. A method according to claim 32-33, wherein the activatable groups of step (a) are carbon-carbon double bonds.

35. A method according to any one of claims 32-34, which also comprises to provide the activatable groups present at the surface of at least one porous particle in a step preceding step (a).

36. A method according to claim 35, wherein the step preceding step (a) comprises to allylate hydroxyl groups present on the surface of a porous support.

37. A method according to any one of claims 35-36, wherein steps (a)-(c) are replaced by a single step wherein an activated ligand is reacted with the activatable groups present on the surface of the support.

38. A method according to any one of claims 32-37, wherein the activation agent used in step (b) is a halogen.

39. A method according to any one of claims 32-38, wherein at least one chemical gradient is a ligand density gradient.

40. A method according to anyone of claims 32-38, wherein at least one chemical gradient is a continuous and smooth gradient.

41. A method according to any one of claims 32-40, wherein the at least two different functionalities are provided in step (c).

42. A method according to claim 41, wherein said at least two different functionalities are provided by one compound.

43. A matrix according to claim 41, wherein said at least two different functionalities are provided by different compounds.

44. A method according to any one of claims 32-43, wherein two or more chemical gradients are provided in the support, one of which is a ligand density gradient.

45. A method according to any one of claims 32-44, wherein the support comprises porous particles, preferably essentially spherical particles, and at least one gradient extends between the centre and the exterior surface of each porous particle.

46. A separation matrix prepared by the method according to any one of claims 20-45.

47. A process of liquid chromatography, wherein a liquid comprising at least one target molecule is contacted with a separation matrix, which comprises ligands coupled to the

surfaces of a porous support, and the target molecule(s) are adsorbed to the matrix, wherein the ligands provide at least one chemical gradient within the support.

48. A process according to claim 47, wherein the liquid is applied in a flow direction which is perpendicular to at least one chemical gradient within the support.

49. A process according to claim 47 or 48, which further comprises a step of eluting the adsorbed target molecule from the matrix by contacting the matrix with an eluent.

50. A process according to any one of claims 47-49, wherein at least one chemical gradient is a ligand density gradient.

51. A process according to any one of claims 47-50, wherein the support comprises essentially spherical porous particles and at least one chemical gradient extends between the centre and the exterior surface of each porous particle.

52. A process according to claim 47-51, wherein the functional groups of the ligands are zwitterions and the elution is performed at a pH different from that during the adsorption.

53. A process according to any one of claims 47-52, wherein the separation matrix is as defined in any one of claims 1-15.